

1 1. A method for determining the concentration of chloride ions in samples,
2 comprising:

3 preparing an enzyme reagent, said enzyme reagent including:

4 α -amylase that is substantially calcium-free; and

5 an α -amylase activity detecting substrate; and

6 combining the enzyme reagent with sodium ion and a sample containing
7 chloride ion to be assayed, the sodium ion being present in a higher concentration
8 than said chloride ion;

9 assaying the quantity of α -amylase formed due to the presence of sodium ions
10 and chloride ions in said sample; and

11 determining the quantity of said chloride ions by reference to said assay of α -
12 amylase.

14 2. The method according to claim 1, wherein calcium is removed from the α -
15 amylase that is substantially calcium-free by use of a chelating compound.

3. The method according to claim 1, wherein calcium is removed from the α -amylase that is substantially calcium-free by use of a compound that forms a covalent bond with calcium.

1 4. The method according to claim 2, wherein said chelating compound is a
2 member selected from the group consisting of ethylenediaminetetraacetic acid, trans-1,2-
3 cyclohexanediamine-N,N,N',N'-tetraacetic acid, glycol ether diamine tetraacetic acid,
4 iminotetraacetic acid, and diaminopropanetetraacetic acid.

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6 5. The method of claim 2, wherein said chelating compound is
7 ethylenediaminetetraacetic acid.

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9 6. The method according to claim 1, wherein said α -amylase activity detecting
10 substrate is a member selected from the group consisting of 2-chloro-4-nitrophenyl- α -D-
11 maltotrioxide, 4-nitrophenyl- α -D-maltopentaoside and α -glucosidase, 2-chloro-4-
12 nitrophenyl- β -D-maltopentaoside and α -glucosidase and β -glucosidase, 4-nitrophenyl- α -D-
13 maltoheptaoside, α -glucosidase, and 2-chloro-4-nitrophenyl- β -D-maltoheptaoside and α -
14 glucosidase and β -glucosidase.

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16 7. The method according to claim 6, wherein said α -amylase activity detecting
17 substrate is 2-chloro-4-nitrophenyl- α -D-maltotrioxide.

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19 8. The method according to claim 1, wherein said sample is a bodily fluid
20 sample.

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22 9. The method according to claim 8, wherein said bodily fluid sample is
23 selected from the group consisting of serum, plasma, or urine.

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- 1 10. The method of claim 1, wherein said sodium ion compound is sodium citrate.
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- 3 11. The method of claim 1, wherein said sodium ion compound is sodium
- 4 acetate.
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1 12. A composition for use in determining the concentration of chloride ions in a
2 fluid sample, comprising: α -amylase that is substantially calcium-free, sodium ion, and an α -
3 amylase activity detecting substrate.

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5 13. A composition as in claim 12 further comprising a compound capable of
6 forming a chelate with a calcium ion and a calcium chelate compound.

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8 14. A composition according to claim 13, wherein said compound capable of
9 forming a chelate with a calcium ion is a member selected from the group consisting of
10 ethylenediaminetetraacetic acid, trans-1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid,
11 glycol ether diamine tetraacetic acid, iminotetraacetic acid, and diaminopropanetetraacetic
12 acid.

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14 15. A composition according to claim 13, wherein said compound capable of
15 forming a chelate with a calcium ion is ethylenediaminetetraacetic acid.

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17 16. The composition according to claim 13, wherein said calcium chelate
18 compound is calcium-ethylenediaminetetraacetic acid.

1 17. The composition according to claim 12, wherein said α -amylase activity
2 detecting substrate is a member selected from the group consisting of 2-chloro-4-
3 nitrophenyl- α -D-maltotrioxide, 4-nitrophenyl- α -D-maltopentaoside and α -glucosidase, 2-
4 chloro-4-nitrophenyl- β -D-maltopentaoside and α -glucosidase and β -glucosidase, 4-
5 nitrophenyl- α -D-maltoheptaoside, α -glucosidase, and 2-chloro-4-nitrophenyl- β -D-
6 maltoheptaoside and α -glucosidase and β -glucosidase.

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8 18. The composition according to claim 12, wherein said α -amylase activity
9 detecting substrate is 2-chloro-4-nitrophenyl- α -D-maltotrioxide.

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11 19. The composition of claim 12, wherein said sodium ion compound is sodium
12 citrate.

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14 20. The composition of claim 12, wherein said sodium ion compound is sodium
15 acetate.

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1 21. A method of activating calcium-free α -amylase for enzymatic activity
2 comprising mixing chloride ion with calcium-free α -amylase in the presence of excess
3 sodium ion.

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1 22. A method for determining the concentration of sodium ions in samples,
2 comprising:

3 preparing an enzyme reagent, said enzyme reagent including:

4 α -amylase that is substantially calcium-free; and

5 an α -amylase activity detecting substrate; and

6 combining the enzyme reagent with excess chloride ion, and a sample
7 containing sodium ion to be assayed, the chloride ion being present in a higher
8 concentration than said sodium ion;

9 assaying the quantity of α -amylase formed due to the presence of sodium ions
10 and chloride ions in said sample; and

11 determining the quantity of said sodium ions by reference to said assay of α -
12 amylase.

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14 23. The method of claim 22, wherein a calcium-binding compound is combined
15 with the enzyme reagent, the excess chloride ion, and the sample containing sodium ion to
16 be assayed before the α -amylase quantity is determined.

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18 24. The method of claim 22, wherein said calcium-binding compound is
19 ethylenediaminetetraacetic acid.

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